CMFRI Special Publication No. 83 ISSN : 0972-2351

MANGROVE ECOSYSTEMS

A MANUAL FOR THE ASSESSMENT OF BIODIVERSITY

A follow up of the
National Agricultural Technology Project
(NATP.), ICAR.

Mangrove Ecosystem Biodiversity :
Its Influence on the Natural Recruitment of
Selected Commercially Important Finfish and Shellfish
Species in Fisheries

Edited by :
Dr. George J. Parayannilam

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Central Marine Fisheries Research Institute
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ISSN : 0972-2351

CMFRI Special Publication No. 83

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Cover design :
Sreejith K. L.

Price :
Indian Rs. 600/-
Foreign $ 60/-

Printed at :
Niseema Printers & Publishers, Cochin - 18. Kerala, India. Ph : 0484-2403760

Physico Chemical Parameters
WATER

P. Kaladharan, A. Nandakumar, K.K. Valsala and Ansy Mathew

1. *GLOBAL POSITIONING SYSTEM (*GPS)

GPS is a space based all weather radio navigation system. Portable, handy model available operated by dry cells. It is also an indispensable device for G.I.S.

GPS provides precise information on

- Geographic position
- Velocity
- Time
- Altitude

GPS can be used in

A.Fixing/locating sampling stations

B.Determination of vegetative cover

C.Plankton collection (Distance of dragging and hauling the net at a known speed).

D. Digital mapping etc

2.*TEMPERATURE*

Temperature is one of the important parameters affecting physico-chemical and biological changes in both water and sediment. Temperature shows diurnal and seasonal variations. Changes in the atmospheric temperature has a direct bearing on the surface of the water body. Hence simultaneous measurement of temperature from atmosphere and water surface is essential.

Water temperature at the surface is measured using a mercury filled Celsius thermometer with a graduation of 0.1º C. A bucket- thermometer is more convenient for measuring surface temperature with ease and accuracy.

Note

Separate thermometers should be used for measuring air and water temperatures. If only one thermometer is available in the field, air temperature must be measured first.

3. LIGHT PENETRATION

Secchi-disc is the commonly used instrument to measure the light penetration to subsurface depths. It is a 20 cm diameter circular metal plate. The upper surface is divided into four equal quadrants, located diagonally and painted alternatively in black and white colour. A staple is fixed at the center of upper surface for attaching a long graduated rope. On the lower surface weight is fixed in proper position incase requires, to facilitate easy immersion paint black colour in the lower side.

Operation:

The disc is lowered vertically into the water by releasing the graduated rope. The depth at which the Secchi-disc disappears is to be noted (d1) Then slowly lift the disc and again note the depth when it reappears(d2). The average of the two readings gives the depth of light penetration (Euphotic zone) which is expressed in cm.

Calculation:

Depth of light penetration D cm = $\frac{d1+ d2}{2}$

The symbol of Secchi disc extintion depth is ZsD

Precaution:

1. Keep Secchi disc surface always clean and bright.

2. Observation should be made when the sky is clear and bright and avoid shadows.

4. HYDROGEN ION CONCENTRATION (pH)

Principle:

The pH of a solution is measured with a pH meter.

pH is the negative logarithem of the hydrogen ion concentration. Hydrogen ion concentration and pH are not the same. The former can be averaged; but pH being a log function should not be averaged. When the electrodes are dipped in two solutions of different pH levels and connected, a potential difference is set up between the two electrodes, which is measured by the Potentiometer. This is directly related to the pH of the solution.

Procedure:

1) Warm up the instrument for 15-20 minutes before use.
2) Calibrate the instrument with the standard buffer solutions, (pH 4, 7 or 9). Calibration is done by a buffer solution with the pH close to that of the sample.
3) Clean the electrode with double distilled water/ deionised water.
4) Immerse the electrode in the sample and stir for 3 minutes and record the pH.

Note:

Bring the sample to room temperature before measuring the pH.

5. *SALINITY*

Introduction:

Salinity is usually estimated by either titrimetric method or using a salinometer. The method explained below is titrimetric.

Principle:

In this method the halogen ions in seawater are titrated with silver nitrate using potassium chromate as indicator. The halogen ions (except fluoride) readily react with silver to give insoluble silver halides. In this method silver will react with chromate only after all the halide ions, other than fluoride, are precipitated and as soon as a slight excess of silver ion is present, red silver chromate is formed. A faint red colour of the solution indicates the end point of the titration. The total quantity of silver nitrate required to react with chloride, bromide and iodide is a measure of the chlorinity of seawater.

Reagents required:

1) Silver nitrate (24.5 gm/l.)
2) Potassium chromate (10%)- 10 gm in 100 ml.
3) Standard seawater

Procedure:

Pipette out 10 ml of Standard seawater into a 250 ml conical flask. Add four drops of potassium chromate solution and using a mechanical stirrer titrate against silver nitrate solution. Repeat for accuracy/confirmation. Pipette out 10 ml of the seawater sample into the conical flask and proceed as above.

Calculation:

$$\text{Salinity of sample} = \frac{V_2 \times S}{V_1}$$

Where

V_1= Volume of silver nitrate for 10 ml standard seawater

V_2= Volume of silver nitrate used for titration of 10 ml sample

S = Salinity of Standard seawater

b) Potentiometric method:

Alternatively salinity of water samples can be measured using electronic instrument with appropriate electrode. Portable and handy instruments are available in the market.

6. *DISSOLVED OXYGEN*

(Winkler method)

Principle:

Winkler method, depends upon the oxidation of manganous dioxide (bivalent manganese) by the oxygen dissolved in the sample resulting in the formation of a tetravalent compound. When the water containing the tetravalent compound is acidified, free iodine is liberated from the oxidation of potassium iodide. The free iodine is chemically equivalent to the amount of dissolved oxygen present in the sample and is determined by titration with a standard solution of sodium thiosulphate.

$$MnSO_4 + 2KOH \rightarrow Mn(OH)_2 + K_2SO_4$$

If the precipitate is white there is very little dissolved oxygen in the sample. A brown precipitate indicates that oxygen is dissolved in it and reacted with the manganous hydroxide to form manganic oxide.

$$2Mn(OH)_2 + O_2 \rightarrow 2MnO(OH)_2$$

On addition of acid, the precipitate is dissolved forming manganic sulphate.

$MnO(OH)_2 + 2H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O$

Due to an immediate reaction between this compound and the potassium iodide added previously, iodine is liberated resulting in the typical iodine colouration of the sample.

$Mn(SO_4)_2 + 2KI \rightarrow MnSO_4 + K_2SO_4 + I_2$

The number of molecules of iodine liberated by the reaction is equivalent to the number of molecules of oxygen dissolved in the sample and this can be determined by titrating against standard solution of sodium thiosulphate using starch as indicator.

$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$

Reagents:

1) Sodium thiosulphate solution (1.25 gms in 1 ltr.)
2) Starch solution – 1gm starch made into a paste with distilled water and diluted to 100 ml, boiled and kept with 1 ml formalin as preservative.
3) Winkler solution A – 20 gms of manganous sulphate in 100 ml water.
4) Winkler solution B – 41 gm of sodium hydroxide + 25 gm of potassium iodide in 100 ml water.
5) Concentrated sulphuric acid.
6) Standard potassium iodate – Accurately weigh 0.1784 gm of potassium iodate into a 1 ltr. volumetric flask and dissolve and make up to the volume - (this is 0.005N)
7) Potassium iodide.

Procedure:

Collect the water sample in a 125 ml glass stoppered bottle without any air bubbles. Take out the stopper and add 1ml each of Winkler A and Winkler B solution. Close the bottle. Shake the bottle gently till the precipitation formed is evenly distributed. Allow settling. Then add 2 ml con. H_2SO_4; close bottle and gently shake till the precipitate is completely dissolved.

Pipette out 10 ml of potassium iodate solution into a conical flask. Add 1 gm of potassium iodide and 2 ml of conc. sulphuric acid. Dilute to 100 ml and titrate against sodium thiosulphate solution till the colour becomes pale yellow. Add 1 ml of starch solution, shake well and continue the titration till the blue colour disappears. Repeat the analysis

Pipette out 100 ml of the preserved sample and titrate against standard sodium thiosulphate as above.

Calculation:

Normality of potassium iodate $= \frac{\text{Weight/litre}}{35.67} = N_1$

Normality of thiosulphate $= \frac{N_1 \times 10}{\text{Titrated volume of thiosulphate for 10 ml of potassium iodate}} = N_2$

Hence amount of dissolved oxygen in ml/ltr. =

$$\frac{\text{ml. Thiosulphate} \times N_2 \times 8 \times 1000 \times R}{100 \times 1.429}$$

Where

R is the correction factor =1.01 i.e. 125/125-2

1.429 is the conversion factor from ppm to ml/lit.

7. *DISSOLVED ORTHOPHOSPHATE*

(Ascorbic acid method)

Introduction:

Phosphorous present in seawater in the form of dissolved orthophosphate can be easily determined quantitatively based on the method given by Murphey and Riley, (1962).

Principle:

Ammonium molybdate and potassium antimony tartrate react in an acid medium with diluted solutions of orthophosphate to form phosphomolybdic acid that is reduced to the intensely coloured molybdenum blue by ascorbic acid. The intensity of the blue colour increases in proportion to the amount of phosphorous present and can be measured photometrically.

Reagents:

1) Sulphuric acid solution 5N: Dilute 70 ml concentrated H_2SO_4 with 500 ml distilled water.
2) Potassium antimony tartrate solution: dissolve 1.3715 g K (SbO) $C_4H_4O_6$. $\frac{1}{2}H_2O$ in 400 ml distilled water in a 500 ml volumetric flask and dilute to volume. Store in a glass stoppered bottle.
3) Ammonium molybdate solution: Dissolve 20 g $(NH_4)_6 Mo_7 O_{24}$. $4H_2O$ in 500 ml distilled water. Store in a plastic bottle at 4º C.
4) Dissolve 1.76 g of ascorbic acid in 100 ml distilled water. This solution is stable.
5) Mixed reagent: Mix the above reagents in the following proportions. For 100 ml combined

reagent, 50 ml 5N H_2SO_4, 5 ml potassium antimony tartrate solution, 15 ml ammonium molybdate solution and 30 ml ascorbic acid solution. Mix after addition of each reagent. The reagent is stable for 4 hrs.

6) Standard stock phosphate solution: Dissolve accurately 0.816 gm of anhydrous potassium dihydrogen phosphate (KH_2PO_4) in 1000 ml of distilled water. Store in dark bottle with 1 ml of chloroform.1 ml of this solution contains 6µg of PO_4-P.

Procedure:

To 50 ml of the sample at laboratory temperature add 8.0 ml of mixed reagent. After 5 minutes and preferably within the first 30 minutes measure the extinction of the solution, in a 1 cm cell against distilled water at a wavelength of 885nm.

Warm another portion of the sample to laboratory temperature and measure the absorption to obtain turbidity correction. Correct the measured extinction of the sample by subtracting both the turbidity and reagent blank.

Preparation of calibration graph:

Dilute the standard stock solution to get working standards of 1.2, 2.4, 4.8, 7.2, 9.6 and 12.0 µg of PO_4-P/ ltr. concentrations. Follow the above procedure and measure the absorbance of the standards at 885nm. Draw a calibration graph.

Calculation:

Obtain the concentration of PO_4-P in the sample from the calibration graph.

Note:

1) Samples are to be collected in polythene bottles and analysis are to be carried out within an hour of collection. If the analysis are delayed then the samples must be frozen.
2) All the reagents must be in the room temperature before they are mixed and must be mixed in the order given. If turbidity forms in the mixed reagent shake and let it stand for a few minutes until the precipitate disappears before proceeding.
3) If the samples are high in phosphate, dilute them with distilled water before the reagents are added.

8. *REACTIVE SILICATE*

Introduction:

Silicon present in seawater in the dissolved form mainly as the alkali salts of orthosilicic acid $Si(OH)_4$, is estimated by the method described by Mullin and Riley (1955) and as modified by Strickland and Parsons (1968).

Principle:

Determination of Silicate in natural waters is based on the principle that yellow silicomolybdic acid is produced when silicomolybdates react with acids. But all forms of silica in solution will not react to give the silicomolybdate complex. Depending on the pH, the silicomolybdate complex exists in two isomeric forms (the alpha and beta silicomolybdic acids). The beta form is very unstable. The alpha form, termed 'reactive silicate' is the most available form, turns into a blue complex on reduction with ascorbic acid, which can be measured photometrically.

Reagents:

1. Acid ammonium molybdate: Shake 2 g of ammonium molybdate with approximately 70 ml of water, add 6 ml of conc. HCl to dissolve the salt completely. Dilute to 100 ml and if necessary filter. Since the reagent takes up silica from glass it should be stored in polythene bottles.
2. Oxalic acid: Dissolve 10 g of oxalic acid dihydrate in water, dilute to 100 ml and filter.
3. Sulphuric acid 25 % v/v
4. Metol-sulphite solution: Dissolve by shaking, 5g of metol in about 240 ml of water, containing 3g of anhydrous sodium sulphite and dilute to 250 ml. The solution, after filtration through a Whatman No.1 filter paper, should be stored in a dark glass bottle.
5. Reducing agent:

   Mix 100 ml of the metol sulphite solution with 60 ml of 10 % oxalic acid and add, while cooling, 120 ml of 25 % H_2SO_4, dilute to 300 ml. The fresh reducing agent should be prepared fortnightly.
6. Standard silicate solution: 0.960 g of sodium silico fluoride is dissolved in distilled water and make up to 1000 ml. 1 ml of this solution contains 5µg-of Si.

Treatment of apparatus:

Graduated flasks should be allowed to stand overnight with a mixture of concentrated nitric acid and sulphuric acids (1:1) to render them insoluble. After this treatment they should be thoroughly washed

with tap water and distilled water. The flasks may be drained, but should not be allowed to become completely dry, as this may render them more soluble.

Method:

Pipette out 20 ml of the sample (up to 2 µg-**of** Si) – (If the sample contains more than 2 µg-of Si take 15 ml of seawater and add about 5 ml of distilled water) - into a 50 ml graduated flask containing 3 ml of the acid molybdate reagent and mix thoroughly. After 10 minutes add 15 ml of reducing agent and make up to 50 ml with distilled water. Allow to stand for 3 hours. Measure the optical density of the solution at 810 nm in a spectrophotometer. Use a reagent blank and set the instrument at 0.0 absorbance.

Preparation of calibration graph:

From the stock solution a series of working standards of known concentrations of silicates are prepared by suitably diluting with distilled water. The diluted working solutions of 2.5, 5.0, 10.0, 25.0, 50.0 and 100.0 µg-**of** Si/ lit. are prepared and treated with reagents and absorbance values are measured at 810 nm. Draw a calibration graph.

Calculation:

Concentration of the reactive silicate in the given sample is obtained from the graph.

Note:

1) Glass bottles must be avoided for sampling or storage; plastic containers are suitable. Because of the possible presence of siliceous organisms, storage in the dark is advised but analysis should in any case not be delayed for more than 24 hours. However if these are unavoidable, freezing of the sample would probably help to minimize changes.
2) For samples of salinity below 27‰, overnight standing after thawing is essential to allow silicon polymerized by freezing to depolymerize.

9. NITRATE

Introduction:

The estimation of Nitrate in seawater is based on a method by Morris and Riley (1963) with some modifications suggested by Grasshoff (1964) and Wood et. al. (1967).

Principle:

Nitrate in seawater is reduced almost quantitatively to nitrite when a sample is run through a column containing cadmium filings coated with metallic copper. The nitrite produced is determined by diazotizing with sulphanilamide and coupling with N- (1-naphthyl)-ethylenediamine to form a highly coloured azodye, which can be measured spectrophotometrically. Any nitrite initially present in the sample should be corrected .

Special apparatus:

A reduction column may be conveniently made.

Reagents:

1) Concentrated Ammonium chloride solution

   Dissolve 125 gm of AR grade ammonium chloride in 500 ml of distilled water and store in a glass or plastic bottle.

2) Dilute Ammonium chloride solution

   Dilute 50 ml of concentrated ammonium chloride solution to 2000 ml with distilled water. Store the solution in a glass or plastic bottle.

3) Cadmium-copper filings

   Cadmium filings of a specific size range are required for the columns. They may be brought or made from cadmium metal by filing the metal with a coarse wood file. Filings should pass through 2 mm mesh size and be retained by 0.5 mm mesh size. Stir about 100 g of fillings (sufficient for 2 columns with 500 ml of 2% w/v solution of $CuSO_4$ ($CuSo_4.5H_2O$) until the blue colour disappear in the solution. Place a small plug of copper wool (turnings) in the bottom of the reduction column and fill the column with diluted ammonium chloride solution.

Pour in slurry of the cadmium-copper filings and gently pack the column to the required height. Do not allow the filings to become dried out during the procedure. They should continue to be covered with diluted ammonium chloride or the seawater samples at all times. Wash the column thoroughly with diluted ammonium chloride and adjust the flow rate by tapping the side of the column so that about 100 ml is collected in 8-12 minutes. If the flow rate is slower than this, the column has to be re-packed. Add a small plug of copper wool to the top of the column.

The cadmium-copper filings may be reactivated after continued use (as judged by the F-value obtained for the standard). Filings are removed from the column, washed with 5% v/v HCl and then washed with distilled water until the pH of the decanted solution is >5. The filings can then be reactivated with

copper sulphate using the procedure given above.

4) Sulphanilamide solution

Dissolve 5 g of sulphanilamide in a mixture of 50 ml of concentrated HCl (sp.gr.1.18) and 300 ml of distilled water. The solution is stable for many months.

5) N- (1-naphthyl)- ethylene diamine dihydrochloride solution

Dissolve 0.5 g of the dihydrochloride in 500 ml of distilled water. Store the solution in a dark bottle. The solution should be preapred afresh once a month or when a strong brown colouration develops.

6) Synthetic seawater

Dissolve 310 g of AR quality sodium chloride (NaCl), 100 g of AR quality magnesium sulphate ($MgSO_4.7H_2O$) and 0.5 gm of sodium bicarbonate ($NaHCO_3.H_2O$) in 10 lit. of distilled water.

7) Standard nitrate solution

Dissolve 1.02 g of AR quality potassium nitrate (KNO_3) in 1000 ml of distilled water. The solution should be stored in dark bottle and is generally stable in the absence of evaporation. Dilute 2.0 ml of this solution to 1000 ml with synthetic seawater.

This working solution should always be prepared afresh before use.

Concentration = 20µ g –of N/l.

Procedure:

Add 2 ml of conc. ammonium chloride to the sample. Transfer 100 ml of the sample into an Erlenmeyer flask. Mix the solution and pour about 5 ml on to the top of the column and allow it to pass through.

Add the remainder of the sample to the column and place the drained Erlenmeyer flask under the collection tube. Collect about 40 ml and discard. Collect about 50 ml in a graduated cylinder and dispense this into the Erlenmeyer flask, which contained the original sample. Allow the column to drain before adding the next 5 ml sample (as above).

To the 50 ml sample, add 1 ml of sulphanilamide solution from an automatic pipette. Mix and allow the reagent to react for more than 2 minute but less than 8 minutes. Add 1 ml of naphthylethylene diamine (NNED) solution and mix immediately. After 10 minutes and within 2 hrs. measure the extinction of the solution in a 1 cm cuvette against distilled water at a wavelength of 545 nm in a spectrophotometer. Correct the observed extinction with that of the reagent blank.

Carry out the procedure given above, using 100 ml of diluted Ammonium chloride instead of the seawater sample. Measure the absorbance using the same cuvette as is used for the samples and subtract the blank value from the sample values for each column.

Also carry out the procedure with 100 ml of the dilute standard nitrate solution. Measure the extinction for each individual column; then the factor F is

$F = 20/E_S$

Where

E_S is the extinction of the standard corrected for the blank.

Calculation:

µg-of N/l = (Corrected extinction x F) – 0.95 C

Where 'C' is the concentration of nitrite in the sample in µ g-of N/l.

Note:

1) If the samples are stored they should be frozen at -20º C. In the presence of high concentrations of phytoplankton, samples should be filtered before analysis.

2) Because of the small salt effect, standard nitrate solutions should be made up in synthetic seawater or a low nitrate seawater sample should be 'spiked' with a standard amount of nitrate.

3) Column dimensions can be scaled down proportionally and smaller seawater samples can be used as required by users.

4) For extinction values of >1.0 or <1.0 use an appropriate cuvette cell length (i.e. 0.5 cm or 10 cm respectively) and adjust the factor which ever is appropriate.

5) In most samples of seawater, the level of nitrite will be insignificant and the correction can be largely ignored. However in some cases, particularly with respect to depth profiles where a nitrite maximum is expected, a correction should

be employed. The factor of 0.95 allows for an approximate 5% loss of nitrite on the column compared with the direct determination.

6) For the blank and standard values, the extinctions obtained should be applied to individual cadmium columns and not averaged. Each column may have small consistent differences that are allowed for only if the blank and standards are applied on an individual basis.

10. NITRITE

Nitrite in water sample is determined by the similar method described for Nitrate determination except for reduction through Cadmium column. Hence reagents and procedure are same as those mentioned for Nitrate determination. Reduction through Cadmium column is not required.

Preparation of calibration graph:

Prepare a standard stock nitrite solution by dissolving 0.345 g of sodium nitrite in 1000 ml of distilled water (I ml = 5µg-of). 1 ml of stock solution is diluted to 100 ml with distilled water. Prepare dilutions of 0.05, 0.2, 0.5, 2.0, and 4.0µg-of/ltr and add the reagents. Measure the absorbance at 545 nm and prepare the calibration graph to obtain the nitrite concentration.

Calculation:

Concentration of the nitrite in the water sample is obtained from the calibration graph.

11. *AMMONIA*

(Phenol hypochlorite method)

Introduction:

For the determination of ammonia in the seawater the method involving indo-phenol blue reaction is well known and the one followed here is that of Zolarzano (1969).

Principle:

In this method phenol and hypochlorite react in an alkaline solution to form phenyl quinone-monoimine, which in turn, react with ammonia to form indophenol. Indophenol gives the solution a blue colour, the intensity of which is propotional to the concentration of ammonia present. Sodium nitroprusside is added to intensify the blue colour. Both ammonia and ammonium are measured, because in a strong alkaline solution all the ammonium is converted to ammonia. This procedure gives an estimate of total ammonia nitrogen.

Reagents required:

1) Phenol-alcohol solution: Dissolve 10 g of reagent grade phenol in 100 ml of 95% v/v ethyl alcohol.
2) Sodium nitroprusside 0.5%: Dissolve 1 g of sodium nitroprusside in 200 ml of water.
3) Alkaline solution: Dissolve 100 g of trisodium citrate and 5 g of sodium hydroxide in 500 ml of water.
4) Sodium hypochlorite solution: Use a solution of commercial hypochlorite, which should be atleast 1.5 N.
5) Oxidising solution: Mix 100 ml of sodium citrate solution (alkaline solution) and 25 ml of hypochlorite solution and use the same day (1:4 ratio- sodium hypo chlorite: alkaline solution).
6) Stock standard solution: 0.100 g of ammonium sulphate (A.R Grade) in 1000 ml of distilled water (1 ml = 1.5 µg of N).

Procedure:

The procedure consists of the successive addition of 2 ml of phenol solution, 2 ml of nitroprusside solution and 5 ml of oxidizing solution to 50 ml of sample mixing thoroughly after each addition. The colour is allowed to develop at room temperature (22-27º C) for 1 hr and the absorbance recorded at 640 nm in a spectrophotometer. Correct the absorbance with that of the reagent blank.

Preparation of calibration graph:

Dilute the standard stock solution to get working standards of 1.5, 3.0, 6.0, 9.0, 12, 15 µg of NH_3-N/ ltr. concentrations. Follow the above procedure and measure the absorbance at a wavelength of 640 nm in a spectrophotometer and draw a calibration graph.

Calculation:

Obtain the concentration of NH_3-N in the sample from the calibration graph.

Note:

1) All the reagents are prepared using ammonia free distilled water.
2) All the glasswares used must be cleaned by washing initially with warm dilute hydro-chloric acid and rinsing thoroughly with distilled water.
3) Filter the water sample prior to analysis through Whatman No: 42, or equivalent filter paper.
4) If the strength of hypochlorite is not satisfactory, a fresh reagent should be used for analysis.

SOLIDS

(Total dissolved solids and Total suspended solids)

Introduction:

Solids represent the portion of the water sample that is not lost upon evaporation. Solids include dissolved organic matter, particulate organic matter, dissolved inorganic matter / substances except gases, the carbon dioxide contained in bicarbonate and particulate inorganic substances.

12. *TOTAL DISSOLVED SOLIDS (TDS)*

Principle:

To measure the total dissolved solids (TDS) concentration, a sample is filtered to remove the particulate matter, the filtrate is evaporated and the residue weighed. The TDS concentration indicates the milligram per liter of dissolved organic and inorganic matter in a sample

Special Apparatus:

Glass fiber filtration apparatus,

Gelman type A/E glass fiber filters or equivalent,

Imhoff cones,

100 ml evaporating dishes,

muffle furnace,

large desiccators

semi micro analytical balance.

Procedure:

Prepare glass fiber filters by soaking them in distilled water for 24 hrs. and then drying. Ignite a clean evaporating dish in a muffle furnace at 550º C for 30 minutes, cool the dish in a desiccator and weigh it. Position a filter holder in a suction flask, place a glass fibre filter on the holder, attach the funnel to the holder and attach the apparatus to a vacuum source. Mix the sample and filter 125-150 ml of it through the glass fiber filter.

Measure 100 ml of the filtrate into the tared evaporating dish with a graduated cylinder. Evaporate the contents of the dish in an oven at 95º C. Increase the oven temperature to 103º C for 1 hr. Cool the dish and residue in a desiccator and weigh.

Calculation:

$$\text{TDS (mg/ltr)} = \frac{(F-T)\ 1000}{V}$$

Where

F= Final weight of Evaporating dish and residue in milligrams

T= Tare weight of evaporating dish in milligrams

V= Sample volume in milliliters

13. *TOTAL SUSPENDED SOLIDS (TSS)*

Principle:

The Total Suspended solids (TSS) can be estimated by weighing the residue retained on the glass fibre filter used in the TDS analysis. The TSS in milligrams per litre is a measure of the particulate matter in suspension.

Procedure:

Prepare a glass fibre filter by soaking them in distilled water for 24 hours and then drying. Dry filters in oven at 80-90º C for 24 hrs. and tare. Pass a 100 ml (or larger) sample through the tared glass fibre filter. Remove the filter with small tongs (do not touch) and dry for 24 hrs. at 80-90º C. Cool the filter in a desiccator and weigh to five decimal places.

Calculation:

$$\text{TSS (mg/l.)} = \frac{(F-T)\ 1000}{V}$$

Where F= Final weight of Filter and residue in milligrams

T= Tare weight of Filter in milligrams

V= Sample volume in millilitres

Note:

The TSS analysis can easily be conducted in conjunction with the TDS analysis.

Tare the filter used in the TDS analysis, determine the quantity of the residue resulting from the filtration of the TDS sample, and calculate TSS.

14. *CHLOROPHYLL PIGMENTS*

Principle:

Chlorophyll bearing organisms present in known volume of water sample is filtered and dissolved in a solvent (Acetone 90% v/v). The pigment content dissolved in unit volume of acetone is measured spectrophotometrically. Since on an average, primary production in the ocean bears a fairly constant relation to the chlorophyll content, measurement of these pigments is also used as an index of productivity.

Requirements:

Glass fiber filter papers, vacuum-filtering unit, measuring jar, centrifuge and centrifuge tubes with cap.

Method:

Water samples collected for chlorophyll pigments must be passed through a coarse filter 0.2 mm mesh to remove zooplankton. Thoroughly mix the sample. A known volume (500 ml) of the sample is filtered through a 47 mm GF/C filter paper. The pigment is extracted by adding 10 ml of 90 % v/v acetone to each filter in a centrifuge tube. Tightly stopper the tube with aluminium foil or plastic cap. The extraction is carried out at low temperature for 20 hours in dark. The extract is centrifuged (6000 rpm for 8 minutes) and the final volume is adjusted to 10 ml with the same solvent. Decant the supernatant into a cuvette and measure the absorbance at the following wavelengths (750, 664, 647 & 630 nm). The amount of pigments in the sample is calculated using the revised formula of Jeffery and Humphrey (1975).

Calculation:

Chlorophyll a = 11.85 x **E** 664 – 1.54 x **E** 647 – 0.08 x E 630

Chlorophyll b = 21.03 x **E** 647 – 5.43 x **E** 664 – 2.66 x E 630

Chlorophyll c = 24.52 x **E** 630 – 1.67 x **E** 664 – 7.6 x E 647

Where

E stands for the absorbance at different wavelengths obtained above and corrected by the 750 nm reading; Chlorophyll a, b and c are the amounts of chlorophyll.

$$\text{Then mg Chlorophyll/ } m^3 = \frac{C \times v}{V \times 10}$$

Where

v = volume of acetone used.

V= volume of sample in litres.

C = Amount of Chlorophylls a, b & c

Note:

Water sample must be frozen if filtration can not be done immediately. While filtration the sample should be mixed thoroughly.

POTENTIOMETRIC METHOD OF DETERMINING WATER AND SEDIMENT CHARACTERISTICS

For the easy and *in situ* measurement of physico-chemical parameters of water and sediment , different models of portable multi parameter meters are available for affordable price. These portable electronic instruments do contain besides the main unit, electrodes/probes for recording parameters such as

- Temperature
- Conductivity
- Salinity/Chlorinity
- PH
- Turbidity
- Dissolved oxygen
- Redox potential

Advantages:

The measured parameters can be stored and recorded through a printer in the laboratory .

The portable instruments ensure speedy, *in situ* and accurate measurement of different parameters of water and sediment

The instruments eliminate the risk of storage , transportation and preservation of large number of samples

These instruments can save the recurring expenditure needed for chemicals, costly reagents and sample containers.